Original Article

Neuronal Protection: Insights from Nerve Growth Factor NGF Gene Expression Analysis in Alzheimer's Disease Management

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Conflict of Interest: None.

ABSTRACT

Background: Dementia encompasses a spectrum of neurological disorders, prominently featuring Alzheimer's disease (AD), which constitutes approximately 70% of all dementia cases. Risk factors include genetic predispositions, such as the ApoE ε4 allele, APP, PSEN1/PSEN2, and lifestyle or health-related factors like depression, smoking, hypertension, and Type II diabetes. The latter is notably associated with conditions like hyperglycemia or insulin resistance, which potentially disrupt cerebral function and precipitate cognitive decline.

Objective: This study focuses on the Nerve Growth Factor (NGF) genes, exploring their role in neuroprotection. Specifically, it examines the expression levels of the NGF gene in diabetic patients with dementia and correlates these findings with neural disturbances as gauged by Mini Mental State Examination (MMSE) scores.

Methods: Blood samples (5 ml each) were collected from 100 participants, comprising 68 cases of diabetic dementia and 32 healthy controls, from Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. The study involved nucleic acid extraction, primer design and optimization, and targeted RT-qPCR for NGF expression analysis. ELISA and MMSE scoring were also employed to assess cognitive impairment.

Results: Analysis revealed significantly lower NGF expression in the diabetic dementia group compared to controls. Specifically, an NGF gene expression decrease correlated with cognitive impairment severity; 80% of participants scored below 9 on the MMSE, indicative of severe dementia. A Pearson's correlation coefficient of 0.494 underscored the relationship between NGF expression and cognitive impairment.

Conclusion: The findings highlight the potential role of NGF genes in the pathogenesis of dementia among individuals with diabetes. Changes in NGF expression may be pivotal in dementia development, suggesting that NGF could serve as a biomarker for the diagnosis and potentially guide therapeutic strategies.

Keywords: Cognitive Impairment, Dementia, Expression Analysis, Neurobiology, Neuroprotection, NGF.

INTRODUCTION

Type II Diabetes Mellitus (Type II DM), the most prevalent form of diabetes, has been linked to a spectrum of cognitive impairments, ranging from mild decrements to severe dementia (1). Dementia encompasses a group of neurological disorders, with Alzheimer's disease (AD) representing approximately 70% of all dementia cases (2). Among the significant risk factors identified are genetic variants, notably the ApoE ε4 allele, APP, PSEN1/PSEN2, as well as environmental and health-related factors such as depression, smoking, hypertension, and diabetes mellitus (3). Central to this discussion is the Nerve Growth Factor (NGF), a member of the neurotrophic factors protein family encoded by the NGF gene. NGF plays a critical role in enhancing insulin sensitivity, a pivotal factor in the development of Type II DM, thus indirectly influencing the risk of diabetes (4). Furthermore, NGF's anti-inflammatory properties are noteworthy, given the established link between chronic inflammation and both diabetes and dementia. By attenuating inflammation, NGF may mitigate the risk associated with these conditions (5).

Research has shown that reduced levels of NGF correlate with increased production of Reactive Oxygen Species (ROS), elevated glucose levels, and the accumulation of amyloid β and tau proteins, factors closely associated with Alzheimer's disease. Additionally, NGF deficits have been linked to an increased risk of obesity-related cardiovascular diseases, such as ischemic heart disease and

peripheral artery disease (6,7). Genome-wide association studies (GWAS) have identified several candidate genes with direct or indirect links to Alzheimer's dementia, among which the NGF gene emerges as a novel potential gene of interest (8).

Neurophysiology of NGF (9)	Neuropathology of NGF (10)
• Insulin-sensitizing, anti-inflammatory, angiogenic, and vasodilatory Properties • Controls important brain functions such as energy homeostasis, hippocampal neurogenesis and Synaptic Activity • Controls neurogenesis and synaptic plasticity • AdipoR1 is expressed primarily in the hippocampus • Significant effect on cognitive functions	• Reduction in the levels of NGF or reduction in the NGF signaling activity can promote the progression of Alzheimer's disease and cause cognitive impairment • Decreased plasma levels of NGF are a risk factor for women with Alzheimer's disease • May have a neuroprotective effect on Alzheimer's disease

The neurotrophin family, comprising NGF, BDNF, NT-4/5, and NT-3, plays diverse roles within both the peripheral nervous system (PNS) and central nervous system (CNS). These neurotrophins are crucial for cell maintenance and survival within neuronal populations. TrkA, the high-affinity receptor for NGF, TrkB for BDNF and NT-4, TrkC for NT-3, and p75NTR, the low-affinity receptor common to all neurotrophins, play functional roles in the PNS and CNS (11). This highlights the interconnection between NGF and cognitive impairments in diabetic individuals, focusing on conditions such as hyperglycemia or insulin resistance.

This study aims to explore the NGF gene, which may play a pivotal role in the intersection of Type II diabetes and dementia. By investigating the expression of the NGF gene in diabetic patients suffering from dementia and correlating it with neural disturbances in relation to their MMSE scores, we seek to understand better the intricate link between these conditions. The objective of this investigation is to elucidate the potential role of the NGF gene in modulating the risk and progression of dementia in individuals with Type II diabetes, thereby contributing to the development of more targeted therapeutic interventions.
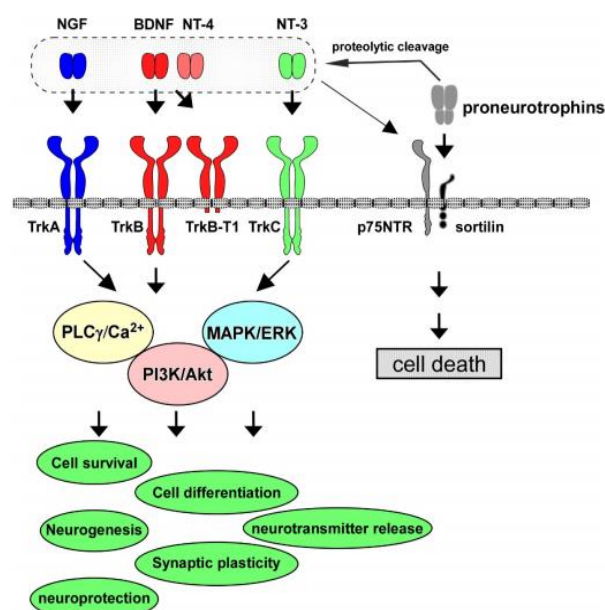

Figure 1. Neurptrophins and Receptors in CNS (11).

MATERIAL AND METHODS

In this study, a total of 100 participants were enrolled, comprising 88 patients diagnosed with Type II Diabetes Mellitus (Type II DM) accompanied by dementia, alongside 12 healthy controls. Following the attainment of written informed consent, blood samples (5ml each) were collected from individuals meeting the study's inclusion criteria: individuals over 40 years of age, of either sex, diagnosed with Type II DM and dementia, and healthy controls. The exclusion criteria were the absence of a clinical history and diagnostic test records, refusal to provide informed consent, and secondary diabetes. The samples were then transported under standard protocols to designated diagnostics laboratories, where they were stored pursuant to a memorandum of understanding (MOU) until further processing.

For the quantification of serum NGF levels, a Chemiluminescent Immunoassay (CLIA) was employed, utilizing a specific ELISA kit designed for the quantitative determination of serum NGF levels in plasma, in accordance with the manufacturer's instructions.

Genomic DNA was isolated from peripheral blood samples using a commercially available kit, following the manufacturer's guidelines closely. The integrity and quality of the isolated DNA were paramount for subsequent analyses. Thus, several techniques, including UV spectrophotometry, fluorometry, and gel electrophoresis, were employed to assess the DNA's concentration, purity, and integrity. Criteria such as optimal absorbance ratios at 260/280 and 260/230 nm, the visualization of intact bands on a gel, and the confirmation of DNA amplifiability through Polymerase Chain Reaction (PCR) were essential for ensuring the suitability of DNA for further experiments. For gel electrophoresis, a 1.5% agarose gel was prepared and electrophoresed at 70 volts for approximately 40 minutes, with the results analyzed using an SS Doc system.

The study also involved bisulfite modification of DNA and Methylation-Specific PCR (MSP) to investigate the methylation status of the NGF gene. A bisulfite conversion kit was utilized for DNA processing, and a specific CpG site within the NGF gene was targeted

for methylation analysis. PCR amplification of bisulfite-treated DNA was conducted using specific primers and conditions optimized for this purpose.

Primer design was a critical step in the experiment, conducted using a serial cloner software and based on consensus CDS sequences from the NCBI database. The specificity of the designed primers was verified using primer-BLAST. Optimization of the primers, including their melting temperatures and amplicon properties, was achieved through gradient PCR, ensuring efficient and specific amplification of the target sequences.

Table 1. Sequences of Designed Primers

NGF Primers Pair	NGF sequence (5/ to 3/) , −74 nt sequence
Froward	TGCTGGCCTAATAGAGTGGCA
Reverse	CTCAGCGCCATGGAAAATGT

Data from the study were analyzed using Graphpad Prism 9.0 software. Demographic information was presented using bar charts, while the frequencies of relative morbid conditions and expression analyses were also displayed graphically. A one-way ANOVA was conducted to assess variance among the samples, with a p-value of >0.05 considered indicative of statistical significance.

RESULTS

In the conducted study, blood samples from 88 participants diagnosed with diabetic dementia were analyzed, comprising 36 males and 52 females, alongside a control group of 12 healthy individuals. The demographic characteristics and clinical parameters of these participants were meticulously recorded, as outlined in the subsequent tables. The cognitive function of the diabetic dementia patients was assessed using the Mini Mental State Examination (MMSE), a standardized tool for evaluating cognitive impairment.

Gender	Cases (%)	Average Age ($\bar{x}$)	Standard Deviation (S)/σ
Male	41	55.4	±8.5
Female	59	59.1	±4.6

Table 2: Demographical summary of confirmed cases of diabetic dementia patients (n=88)

Clinical Parameters/Variables	Healthy Controls (n=12)	Diabetic Dementia Patients (88)	t-test P value
Fasting Glucose (mmol/L)	5.01± 0.56	9.01± 0.86	0.007*
HbA1c Levels (%)	4.31±0.67	10.59±1.62	0.044*
NGF (µg/mL)	11.37±3.64	4.37±1.38	0.014*
MMSE Scores	24.1±3.12	12.26±4.56	0.009*
*Statistically Significant			

Table 3. Biochemical Parameters of Healthy and Diabetic Dementia Patients (n=100)

The MMSE scores served as a basis for categorizing the severity of cognitive impairment among the participants. According to the scoring criteria established by the creators of the MMSE, scores ranging from 0 to 15 indicated severe cognitive impairment, while scores of 24 or higher suggested minimal to no cognitive impairment. This classification allowed for an in-depth analysis of the correlation between Nerve Growth Factor (NGF) levels and cognitive status among the study groups.

Relative Fold Change of NGF (CH3-DNA) in Diabetic Dementia Patients with Reference to Healthy Controls via RT-qPCR: Upon analyzing the data, it was observed that diabetic patients exhibited significantly lower NGF levels compared to the healthy control group. Furthermore, these lower levels of NGF were associated with decreased

Figure 2: NGF Assessment for Diabetic Dementia and MMSE analysis for Cognitive Impairment.

MMSE scores, reinforcing the link between diminished NGF concentrations and the presence of cognitive impairment in individuals

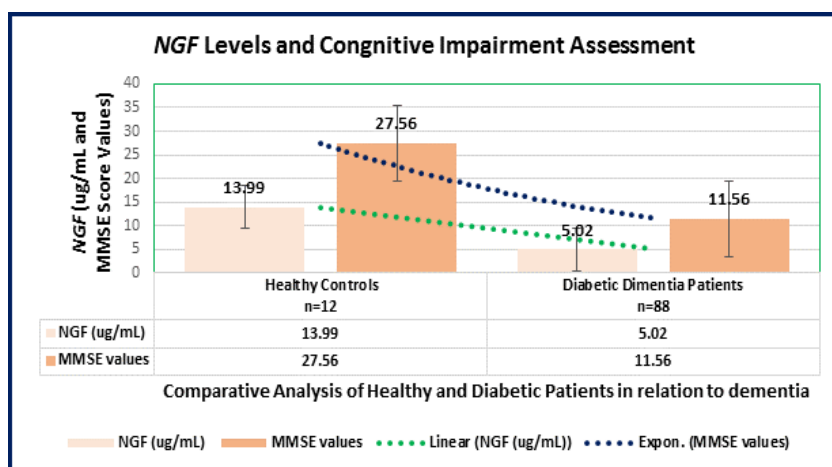with diabetic dementia. The findings underscore the potential role of NGF as a biomarker for cognitive decline in this patient population, highlighting a trend where higher NGF expression was noted in healthy individuals, in contrast to those with diabetic dementia, where NGF levels decreased in tandem with increased cognitive impairment.

DISCUSSION

The interrelation between Type II Diabetes Mellitus (Type II DM) and cognitive impairments, particularly dementia, underscores a complex pathology not limited to mere metabolic dysregulation but extending to neurovascular complications and neurodegeneration. Type II DM

Figure 3: The above figure gives a fold change in healthy controls and diabetic dementia cases.

is known to exacerbate micro and macrovascular complications, thereby affecting neurovascular coupling and elevating the risk of cognitive disorders, including dementia (12). Conditions such as hyperglycemia and insulin resistance are pivotal in increasing the vulnerability to cognitive deficits, emphasizing the significant role of the Nerve Growth Factor (NGF) gene in these processes. This study highlights the correlation between NGF gene expression and cognitive impairment in diabetic dementia, suggesting that alterations in NGF could exacerbate hyperglycemia and contribute to cognitive decline (13).

Our findings reveal a notable decrease in NGF expression among diabetic individuals with dementia, further corroborated by elevated HbA1c levels, indicating poor glycemic control (14). This underexpression of NGF, associated with an increased production of amyloid precursor protein (AβPP) and amyloid β (Aβ) levels, aligns with previous research indicating a potential mechanistic link between NGF dysregulation and Alzheimer's disease (AD) pathology (15,16). The study demonstrated a significant fold decrease in NGF gene expression in subjects with dementia, with even greater reductions observed in those with both diabetes and dementia, suggesting a compounded effect of these conditions on NGF expression (17,18).

The association between reduced NGF levels and cognitive decline, evidenced by diminished serum NGF levels and lower NGF expression in peripheral blood mononuclear cells (PBMCs), underscores the potential of NGF as a peripheral biomarker for dementia (19,20). These findings not only augment our understanding of the pathophysiological mechanisms underlying dementia in the context of Type II DM but also highlight the neuroprotective role of NGF, suggesting that its dysregulation contributes to neurodegenerative processes.

This study's strength lies in its comprehensive analysis of NGF gene expression in the context of diabetic dementia, providing valuable insights into the molecular underpinnings of cognitive impairment in Type II DM. However, the research is not without limitations. The study's cross-sectional design precludes causal inferences, and the relatively small sample size may limit the generalizability of the findings. Furthermore, the study primarily focuses on NGF without considering the complex interplay of other neurotrophic factors and genetic variants that may influence cognitive outcomes in diabetes.

Future research should adopt longitudinal designs to elucidate the causal relationships between NGF dysregulation, diabetes, and dementia. Additionally, exploring the therapeutic potential of targeting NGF signaling pathways may offer new avenues for mitigating cognitive decline in diabetic patients. In conclusion, this study underscores the critical role of NGF in the nexus of diabetes and dementia, offering promising directions for early detection and intervention strategies in managing cognitive impairments associated with Type II DM.

CONCLUSION

This investigation underscores the critical role of the Nerve Growth Factor (NGF) gene as a potential biomarker for the early detection and diagnosis of dementia in patients with diabetes. The downregulation of NGF mRNA in peripheral blood mononuclear cells (PBMCs) suggests a systemic link to neurodegenerative changes, emphasizing the utility of peripheral markers in mirroring central nervous system alterations. Identifying NGF expression alterations early could significantly enhance life expectancy by facilitating the development of targeted therapeutic interventions, thus alleviating the economic and health burden of neurodegenerative diseases on society.
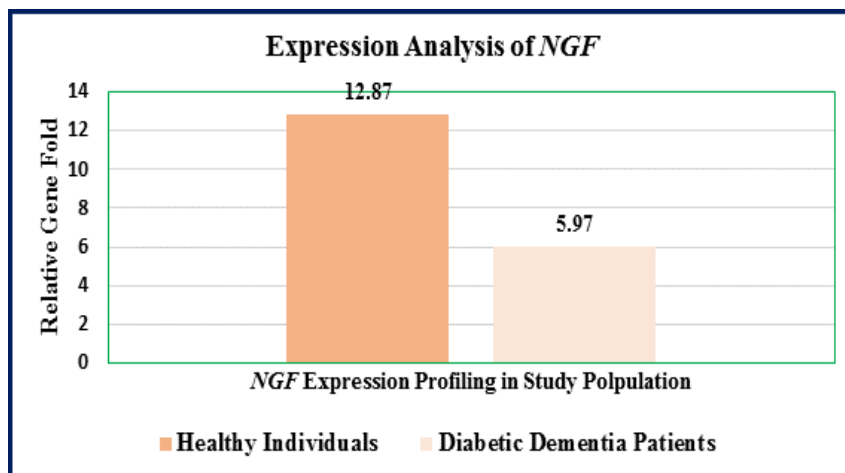
REFERENCES

1. Saedi, E., Gheini, M. R., Faiz, F., & Arami, M. A. (2016). Diabetes mellitus and cognitive impairments. World journal of diabetes, 7(17), 412–422.

2. Quan, M., Cao, S., Wang, Q., Wang, S., & Jia, J. (2023). Genetic Phenotypes of Alzheimer's Disease: Mechanisms and Potential Therapy. Phenomics (Cham, Switzerland), 3(4), 333–349

3. Islam, S., Sun, Y., Gao, Y., Nakamura, T., Noorani, A. A., Li, T., Wong, P. C., Kimura, N., Matsubara, E., Kasuga, K., Ikeuchi, T., Tomita, T., Zou, K., & Michikawa, M. (2022). Presenilin Is Essential for ApoE Secretion, a Novel Role of Presenilin Involved in Alzheimer's Disease Pathogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience, 42(8), 1574–1586.

4. Ochalek, A., Mihalik, B., Avci, H. X., Chandrasekaran, A., Téglási, A., Bock, I., Giudice, M. L., Táncos, Z., Molnár, K., László, L., Nielsen, J. E., Holst, B., Freude, K., Hyttel, P., Kobolák, J., & Dinnyés, A. (2017). Neurons derived from sporadic Alzheimer's disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. Alzheimer's research & therapy, 9(1), 90.

5. Quan, M., Cao, S., Wang, Q., Wang, S., & Jia, J. (2023). Genetic Phenotypes of Alzheimer's Disease: Mechanisms and Potential Therapy. Phenomics (Cham, Switzerland), 3(4), 333–349.

6. Samario-Román, J., Larqué, C., Pánico, P., Ortiz-Huidobro, R. I., Velasco, M., Escalona, R., & Hiriart, M. (2023). NGF and Its Role in Immunoendocrine Communication during Metabolic Syndrome. International journal of molecular sciences, 24(3), 1957.

7. Hölscher C. (2019). Insulin Signaling Impairment in the Brain as a Risk Factor in Alzheimer's Disease. Frontiers in aging neuroscience, 11, 88.

8. Abubakar, M. B., Sanusi, K. O., Ugusman, A., Mohamed, W., Kamal, H., Ibrahim, N. H., Khoo, C. S., & Kumar, J. (2022). Alzheimer's Disease: An Update and Insights Into Pathophysiology. Frontiers in aging neuroscience, 14, 742408.

9. Do Carmo, S., Kannel, B., & Cuello, A. C. (2021). The Nerve Growth Factor Metabolic Pathway Dysregulation as Cause of Alzheimer's Cholinergic Atrophy. Cells, 11(1), 16.

10. Pentz, R., Iulita, M. F., Ducatenzeiler, A., Bennett, D. A., & Cuello, A. C. (2021). The human brain NGF metabolic pathway is impaired in the pre-clinical and clinical continuum of Alzheimers disease. Molecular psychiatry, 26(10), 6023–6037.

11. Abubakar, M. B., Sanusi, K. O., Ugusman, A., Mohamed, W., Kamal, H., Ibrahim, N. H., Khoo, C. S., & Kumar, J. (2022). Alzheimer's Disease: An Update and Insights Into Pathophysiology. Frontiers in aging neuroscience, 14, 742408.

12. Barloese, M. C. J., Bauer, C., Petersen, E. T., Hansen, C. S., Madsbad, S., & Siebner, H. R. (2022). Neurovascular Coupling in Type 2 Diabetes With Cognitive Decline. A Narrative Review of Neuroimaging Findings and Their Pathophysiological Implications. Frontiers in endocrinology, 13, 874007.

13. Spinelli, M., Fusco, S., & Grassi, C. (2019). Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline. Frontiers in neuroscience, 13, 788.

14. Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomarker insights, 11, 95–104.

15. Rajmohan, R., & Reddy, P. H. (2017). Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. Journal of Alzheimer's disease : JAD, 57(4), 975–999.

16. Rajmohan, R., & Reddy, P. H. (2017). Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. Journal of Alzheimer's disease : JAD, 57(4), 975–999.

17. Bruno, F., Abondio, P., Montesanto, A., Luiselli, D., Bruni, A. C., & Maletta, R. (2023). The Nerve Growth Factor Receptor (NGFR/p75NTR): A Major Player in Alzheimer's Disease. International journal of molecular sciences, 24(4), 3200.

18. Shen, L. L., Li, W. W., Xu, Y. L., Gao, S. H., Xu, M. Y., Bu, X. L., Liu, Y. H., Wang, J., Zhu, J., Zeng, F., Yao, X. Q., Gao, C. Y., Xu, Z. Q., Zhou, X. F., & Wang, Y. J. (2019). Neurotrophin receptor p75 mediates amyloid β-induced tau pathology. Neurobiology of disease, 132, 104567.

19. Bradshaw, R. A., Pundavela, J., Biarc, J., Chalkley, R. J., Burlingame, A. L., & Hondermarck, H. (2015). NGF and ProNGF: Regulation of neuronal and neoplastic responses through receptor signaling. Advances in biological regulation, 58, 16–27.

20. Dedoni, S., Olianas, M. C., Ingianni, A., & Onali, P. (2014). Type I interferons up-regulate the expression and signalling of p75 NTR/TrkA receptor complex in differentiated human SH-SY5Y neuroblastoma cells. Neuropharmacology, 79, 321–334.